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Differential sensitivity to the acute psychotomimetic effects of delta-9-tetrahydrocannabinol associated with its differential acute effects on glial function and cortisol

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Abstract

Background. Cannabis use has been associated with psychosis through exposure to delta-9-tetrahydrocannabinol (Δ9-THC), its key psychoactive ingredient. Although preclinical and human evidence suggests that Δ9-THC acutely modulates glial function and hypothalamic-pituitary-adrenal (HPA) axis activity, whether differential sensitivity to the acute psychotomimetic effects of Δ9-THC is associated with differential effects of Δ9-THC on glial function and HPA-axis response has never been tested.

Methods. A double-blind, randomized, placebo-controlled, crossover study investigated whether sensitivity to the psychotomimetic effects of Δ9-THC moderates the acute effects of a single Δ9-THC dose (1.19 mg/2 ml) on myo-inositol levels, a surrogate marker of glia, in the Anterior Cingulate Cortex (ACC), and circadian cortisol levels, the key neuroendocrine marker of the HPA-axis, in a set of 16 healthy participants (seven males) with modest previous cannabis exposure.

Results. The Δ9-THC-induced change in ACC myo-inositol levels differed significantly between those sensitive to (Δ9-THC minus placebo; $M = -0.251$, S.D. = 1.242) and those not sensitive ($M = 1.615$, S.D. = 1.753) to the psychotomimetic effects of the drug ($t(14) = 2.459$, $p = 0.028$). Further, the Δ9-THC-induced change in cortisol levels over the study period (baseline minus 2.5 h post-drug injection) differed significantly between those sensitive to (Δ9-THC minus placebo; $M = -275.4$, S.D. = 207.519) and those not sensitive ($M = 74.2$, S.D. = 209.281) to the psychotomimetic effects of the drug ($t(13) = 3.068$, $p = 0.009$). Specifically, Δ9-THC exposure lowered ACC myo-inositol levels and disrupted the physiological diurnal cortisol decrease only in those subjects developing transient psychosis-like symptoms.

Conclusions. The interindividual differences in transient psychosis-like effects of Δ9-THC are the result of its differential impact on glial function and stress response.

Introduction

Cannabis use is a risk factor for psychosis (Colizzi & Bhattacharyya, 2020; Schoeler et al., 2016a, b). Its main psychoactive ingredient delta-9-tetrahydrocannabinol (Δ9-THC) impacts the endocannabinoid system through its partial agonist effect at the cannabinoid receptor type 1 (CB1) in the brain (Pertwee, 2008). Δ9-THC can stimulate neuronal firing of mesolimbic dopamine neurons and elevate striatal dopamine levels (Sami, Rabiner, & Bhattacharyya, 2015), possibly via CB1-mediated dysregulation of glutamate signaling (Colizzi et al., 2019a), resulting in a psychosis-like state (Colizzi, Weltens, McGuire, Van Oudenhove, & Bhattacharyya, 2019b).

In addition to their predominant neuronal location, CB1 receptors are also located on glial cells, particularly astrocytes (Stella, 2010). Glia have a prominent role in a number of brain processes, including maintaining homeostasis and modulating synaptic activity, immune response, and nervous system restoration after an insult (Garcia-Segura & McCarthy, 2004; Kurosinski & Götz, 2002). Preclinically, chronic cannabinoid-induced CB1 receptor activation has been shown to disrupt glial function (Rubino et al., 2009), in turn affecting glutamate signaling and behavior (Han et al., 2012). Myo-inositol is an astroglial marker whose levels,

especially in the medial prefrontal/anterior cingulate cortex (ACC), have also been shown to be reduced in psychosis patients (Das et al., 2018) and otherwise healthy cannabis users (Blest-Hopley et al., 2019; Prescot, Locatelli, Renshaw, & Yurgelun-Todd, 2011). Glial cells in the medial prefrontal cortex have also been shown to be involved in behavioral responses to stress and in determining differential susceptibility to stress (Bonnefil et al., 2019).

Rodent research and human studies also support a role for the endocannabinoid system in regulating hypothalamic-pituitary-adrenal (HPA) axis activity (Appiah-Kusi et al., 2016), with downstream effects on the developing brain as well as neuronal plasticity in the adult brain (Jauregui-Huerta et al., 2010). In preclinical studies, exogenous cannabinoid administration results in the release of corticotrophin-releasing hormone (CRH), initiating a cascade of events along the HPA-axis, culminating in the release of glucocorticoids from the adrenal cortex into the bloodstream (Pagotto, Marsicano, Cota, Lutz, & Pasquali, 2006). Similarly, acute exposure to Δ-9-THC in healthy individuals induces a dose-dependent increase in cortisol plasma levels (Ranganathan et al., 2009). Glucocorticoids act through glucocorticoid receptors to modulate glutamate signaling (Hillard, Beatka, & Sarvaideo, 2016) and behavior (Jauregui-Huerta et al., 2010). HPA axis dysregulation with higher baseline cortisol levels and blunted cortisol reactivity to acute stress has been reported in psychosis (Borges, Gayer-Anderson, & Mondelli, 2013) and, less consistently, in chronic cannabis users (Cservenka, Lahanas, & Dotson-Bossert, 2018). Emerging evidence also suggests a role for blunted cortisol reactivity in exacerbating anxiety responses to social stress in people at risk of psychosis (Appiah-Kusi et al., 2020).

Even though perturbations of the glial and glucocorticoid (HPA-axis) systems have been reported in psychosis (Jauregui-Huerta et al., 2010) and in cannabis use (Blest-Hopley et al., 2019; Cservenka et al., 2018; Prescot et al., 2011), their biological relevance for the psychosis-inducing effects of Δ-9-THC remains unclear. Despite a role for glial-glucocorticoid interactions in the pathological or neuroprotective responses to stressful insults (Pearson-Leary, Osborne, & McNay, 2015), with glial cells critically regulating stress responses (Pearson-Leary et al., 2015) and glucocorticoids, in turn, acting on glial cells (Jauregui-Huerta et al., 2010), the precise nature of the interaction between these two systems remains unclear, especially in experimental conditions (Jauregui-Huerta et al., 2010). Characterizing them in the same subjects may help elucidate how their alteration may relate to differential sensitivity to the emergence of a Δ-9-THC-induced transient psychosis-like state.

Not everyone experiences psychotic symptoms under the influence of Δ9-THC (Colizzi et al., 2019b) and differential sensitivity to its acute psychotomimetic effects may be moderated by genetic (Bhattacharyya et al., 2012) and neurophysiological factors (Bhattacharyya et al., 2018). Although preclinical and human evidence suggests that Δ9-THC acutely modulates glial function (Han et al., 2012) and levels of cortisol (Ranganathan et al., 2009), the key neuroendocrine marker of the HPA-axis, whether interindividual differences in sensitivity to the acute psychotomimetic effects of Δ9-THC is associated with differential effects of Δ9-THC on glial function and HPA-axis response has never been tested. Using data from our previous study reporting the acute effects of Δ9-THC on psychotomimetic symptoms (Colizzi et al., 2019b) and brain glutamate levels (Colizzi et al., 2019a), we examined whether sensitivity to the acute and transient psychotomimetic effects of Δ9-THC is moderated by the acute effects of a single dose of Δ9-THC on myo-inositol levels as well as time-course of change in day-time cortisol levels. Myo-inositol levels were measured in the ACC, the key brain region where myo-inositol alterations have been associated with cannabis use (Blest-Hopley et al., 2019; Prescot et al., 2011) and psychosis (Das et al., 2018). As medial prefrontal/ACC myo-inositol is found to be reduced in psychosis (Das et al., 2018) and cannabis use (Blest-Hopley et al., 2019; Prescot et al., 2011), and medial prefrontal glial cells mediate stress response (Bonnefil et al., 2019), we predicted that Δ9-THC administration will result in a more pronounced myo-inositol reduction in those sensitive to compared to those not sensitive to the acute psychotomimetic effects of Δ9-THC. As Δ9-THC administration induces a cortisol increase (Ranganathan et al., 2009) and higher cortisol levels associated with a blunted cortisol reactivity to stress have been reported in psychosis (Borges et al., 2013) and cannabis use (Cservenka et al., 2018), we further predicted Δ9-THC administration to result in a more blunted decline in morning serum cortisol levels in those sensitive to compared to those not sensitive to the psychotomimetic effects of Δ9-THC.

Methods

Procedure and participants

The experimental procedure, psychopathological assessment, image acquisition, magnetic resonance spectroscopy (1H-MRS) quantification, and statistics have been previously detailed (Colizzi et al., 2019a; b). Briefly, we employed a double-blind, randomized, placebo-controlled, repeated-measures, within-subject design, with counterbalanced order of drug administration, using an established protocol (Bhattacharyya et al., 2012; Colizzi et al., 2019a). Sixteen right-handed, English-speaking, healthy (confirmed by physical examination) participants (seven males), with no personal or family history of psychiatric illness in first-degree relatives and no history of alcohol abuse, nicotine dependence, or illicit drug use, were assessed on two different occasions separated by at least a 2-week interval, with each session preceded by intravenous administration of Δ9-THC (1.19 mg/2 ml) or placebo. A dose of 1.19 mg was used, as previous work has suggested that an intravenous dose range 0.015–0.03 mg/kg body weight is consistently associated with the induction of psychotomimetic symptoms (Radhakrishnan, Wilkinson, & D'Souza, 2014). Prior to each study visit, participants were advised to remain fast and get at least 6–8 h sleep overnight, and to refrain from smoking for 4 h, to take caffeine for 12 h, and alcohol for 24 h. Also, they had been abstinent from cannabis for at least 6 months before the first study visit and were advised to abstain from using any substance throughout the duration of the study. Abstinence was confirmed on each study day by a negative urinary drug screen for most commonly used drugs. All female participants had a negative pregnancy test; also, all of them were consistently using a reliable contraceptive method, apart from a single subject who underwent both study visits in the first week of the menstrual cycle. Blood samples for serum cortisol measurements (2 ml) were collected in serum-separating tubes at the beginning of the study visit (~9.30 a.m.) and at 20 min (~10.50 a.m.) and 2.5 h (~13.00 p.m.) after drug administration (~10.30 a.m.). Similarly, psychopathological ratings were recorded by an expert clinical researcher, using the Positive and Negative Syndrome Scale (PANSS), a well-established scale used for measuring symptom severity of individuals with psychosis (Kay, Fiszbein, &

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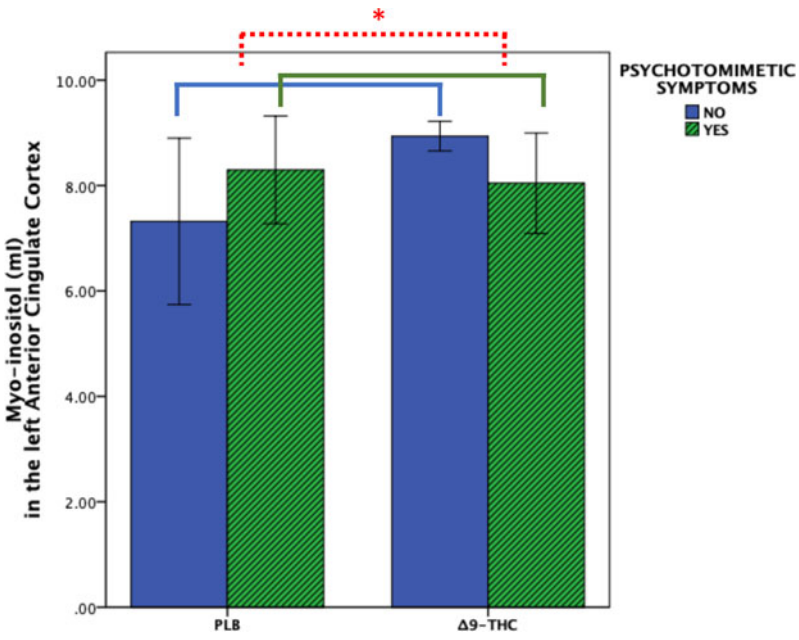

Fig. 1. Acute effect of Δ9-THC on myo-inositol measures in the left Anterior Cingulate Cortex as a function of sensitivity to psychotomimetic effects. The effect of drug administration on myo-inositol levels was obtained subtracting myo-inositol levels post-placebo injection from myo-inositol post-Δ9-THC injection (Δ9-THC minus placebo); the effect of the drug was then compared between individuals sensitive to the psychotomimetic effects of Δ9-THC and individuals not sensitive to the psychotomimetic effects of Δ9-THC by independent *t* test; *, significant effect at $p < 0.05$; error bars show mean and standard deviations; PLB, placebo; Δ9-THC, delta-9-tetrahydrocannabinol.

Opler, 1987), at the same time points before and after drug challenge. Starting at 11.00 a.m., 1H-MRS spectra (Point RESolved Spectroscopy – PRESS; TE = 30 ms; TR = 3000 ms; 96 averages) were acquired on a 3 Tesla MR system in the left caudate head, ACC, and hippocampus, employing the standard GE PROBE (proton brain examination) sequence with CHESS (Chemically Selective Suppression) water suppression, and analyzed with LCModel version 6.3-1L. Metabolite levels were corrected for voxel tissue content. Voxel segmentation and spectral quality have been reported before as well as the values scaled to creatine (Colizzi et al., 2019a). A detailed description of the image acquisition and 1H-MRS quantification is provided in the Supplementary methods.

Statistical analyses

Sensitivity to the psychotomimetic effects of Δ9-THC was based on the manifestation of clearly detectable primary symptoms of psychosis (⩾2-point increase in PANSS delusions, hallucinations, unusual thought content, suspiciousness, and grandiosity items), as drawn from previous factor analytic work (Fulford et al., 2014) as well as previous work to characterize acute sensitivity to Δ9-THC (Bhattacharyya et al., 2018). Data were normally distributed. Repeated measures ANOVA was used to estimate the main effect of Δ9-THC on myo-inositol as well as changes in cortisol levels from baseline to the post-drug injection time points. Independent *t* tests were used to estimate whether the effect of Δ9-THC on myo-inositol levels as well as changes in cortisol levels from baseline to the post-drug injection time points differed between subjects sensitive to and those not sensitive to the psychotomimetic effects of Δ9-THC. A correlation analysis was used to explore the association between ACC myo-inositol and cortisol levels.

Ethics

The study was approved by the Joint South London and Maudsley (SLaM) and Institute of Psychiatry, Psychology & Neuroscience (IoPPN) National Health Service Research Ethics Committee (PNM/13/14-38), and the investigators had a license to use Δ9-THC for research purposes.

Results

Study participants and psychotomimetic effects of Δ9-THC

Study participants had a mean age of 24.44 (S.D.: 4.29) years. All except three (with self-described mixed ethnic origin) of the volunteers were white Europeans. They had 16.94 ± 2.84 years (M ± S.D.) of education.

As expected, administration of Δ9-THC was associated with acute induction of transient psychotic symptoms. Qualitative (Colizzi et al., 2019b) and quantitative (Colizzi et al., 2019a) descriptions of psychotic symptoms under the influence of Δ9-THC in the study sample have been extensively reported before. Eleven subjects (69%) were identified as sensitive to the psychotomimetic effects of Δ9-THC as determined on the basis of ⩾2 point increase in the relevant PANSS items (as described in Methods) (Colizzi et al., 2019a). They had a 5.91 (±4.18) point increase in the primary symptoms of psychosis compared to a 0.6 point increase (±0.55) for the remaining subjects (drug effect, t (14) = 4.13, $p = 0.002$).

Myo-inositol and cortisol levels as a function of sensitivity to the psychotomimetic effects of Δ9-THC

As previously reported (Colizzi et al., 2019a), there was no main effect of acute drug administration on myo-inositol levels in the left ACC. However, the Δ9-THC-induced change in myo-inositol levels differed significantly between those sensitive to (Δ9-THC minus placebo; $M = -0.251$, S.D. = 1.242) and those not sensitive ($M = 1.615$, S.D. = 1.753) to the psychotomimetic effects of the drug ($t(14) = 2.459$, $p = 0.028$; Fig. 1). In those sensitive to the psychotomimetic effects of Δ9-THC, there was a decrease in myo-inositol levels following Δ9-THC administration compared to the placebo condition, while there was an opposite effect of Δ9-THC administration on myoinositol levels in those not experiencing the psychotomimetic effects.

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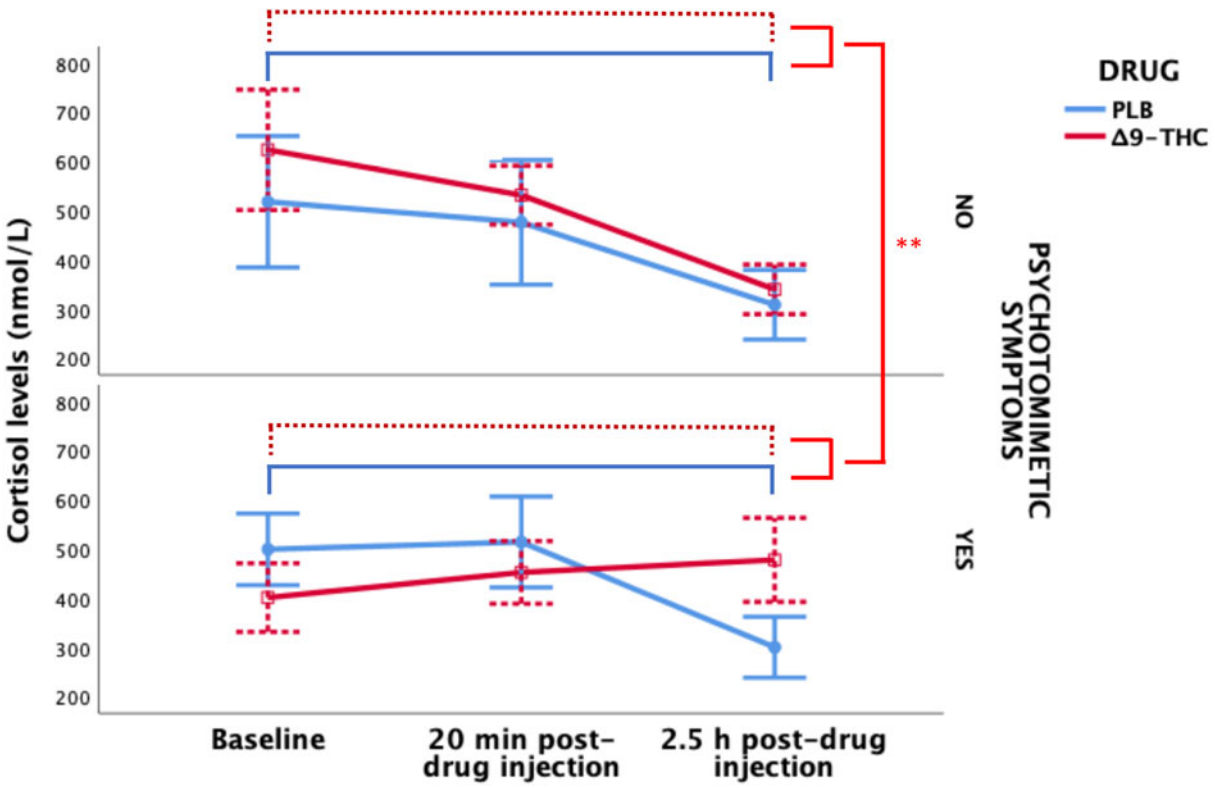

Fig. 2. Acute effect of Δ9-THC on cortisol levels over time as a function of sensitivity to psychotomimetic effects. Change in cortisol levels over time was obtained subtracting cortisol levels 2.5 h post-drug injection from cortisol levels at baseline (baseline minus 2.5 h post-drug injection); the effect of drug administration on change in cortisol levels over time was obtained subtracting the change in cortisol levels over time in the placebo condition from the change in cortisol levels over time in the Δ9-THC condition (Δ9-THC change minus placebo change); the effect of the drug was then compared between individuals sensitive to the psychotomimetic effects of Δ9-THC and individuals not sensitive to the psychotomimetic effects of Δ9-THC by independent *t* test; **, significant effect at $p < 0.01$; error bars show mean and standard errors; PLB, placebo; Δ9-THC, delta-9-tetrahydrocannabinol.

As expected, compared to placebo (baseline minus 2.5 h post-drug injection; $M = 202.33$, S.D. = 138.43), Δ9-THC administration ($M = 43.47$, S.D. = 251.75) resulted in a more blunted cortisol decrease over the study period ($F(1, 14) = 5.463$, $p = 0.035$). Further, compared to placebo, the Δ9-THC-induced change in cortisol levels over the study period (baseline minus 2.5 h post-drug injection) differed significantly between those sensitive to (Δ9-THC change minus placebo change; $M = -275.4$, S.D. = 207.519) and those not sensitive ($M = 74.2$, S.D. = 209.281) to the psychotomimetic effects of the drug ($t(13) = 3.068$, $p = 0.009$; Fig. 2).

As sensitivity to the Δ9-THC-induced transient psychosis-like symptoms seemed to be associated with a reduction of ACC myo-inositol levels as well as a disruption of the physiological decrease of cortisol over time, we further examined their association. We found that the lower the ACC myo-inositol values under Δ9-THC the more blunted was the cortisol decrease over time following Δ9-THC administration (Δ9-THC change minus placebo change), (*rS*:0.468; $p = 0.039$; Fig. 3).

Discussion

To the best of our knowledge, this is the first human study to investigate whether sensitivity to the acute psychotomimetic effects of Δ9-THC moderates its acute effects on both myo-inositol and cortisol levels under experimental conditions. After a single dose of Δ9-THC, subjects developing transient psychosis-like symptoms had lower myo-inositol levels in the ACC and a disruption of the physiological cortisol decrease with cortisol levels remaining even higher for a prolonged time. Also, such Δ9-THC-induced alterations appeared to be interrelated.

Glutamate signaling has been previously shown to be disrupted following acute Δ9-THC administration (Colizzi et al., 2019a; Mason et al., 2019), extending data of chronic cannabis exposure (Colizzi, McGuire, Pertwee, & Bhattacharyya, 2016) and providing a mechanistic neurochemical explanation underlying the acute psychotomimetic effects of Δ9-THC (Colizzi et al., 2019a) that resemble those observed in patients with psychosis (Colizzi et al., 2019b). Glial cells have been involved in inflammation, homeostasis, neurotransmission, and signal transduction, including glutamate metabolism (Verkhratsky, Steardo, Parpura, & Montana, 2016). Consistent, experimental preclinical evidence indicates that Δ9-THC can impair synaptic function via a CB1-mediated reduction of glutamate uptake by glutamate transporters in astrocytes (Chen et al., 2013), resulting in sustained elevation and accumulation of extracellular glutamate (Han et al., 2012). Also, complementary independent human evidence suggests lower levels of myo-inositol in the context of chronic cannabis use directly correlating with glutamate levels, where such relationship is absent in non-using subjects (Blest-Hopley et al., 2019). It is therefore biologically plausible that glia cells might exert a modulating role in the psychotomimetic effects of cannabis. Consistent with this, these results suggest

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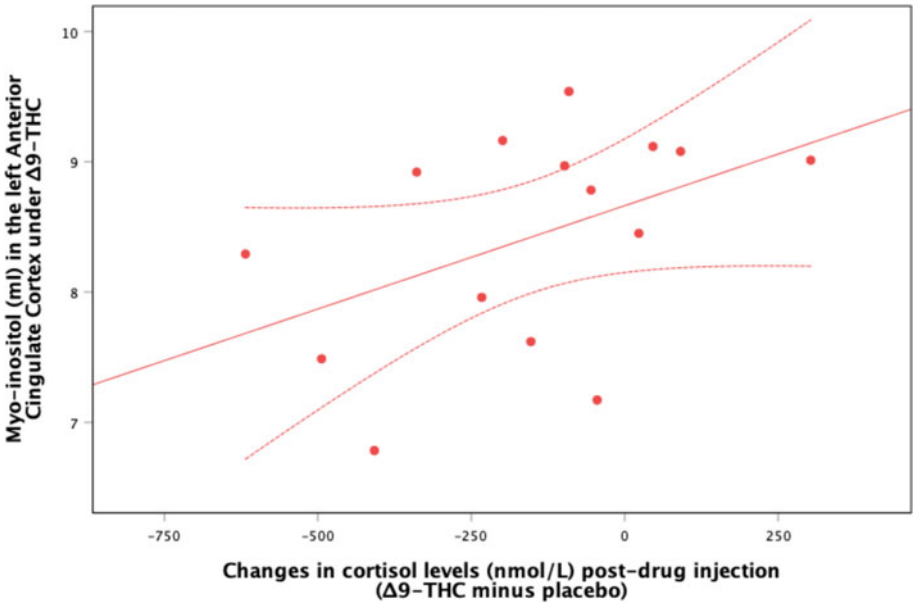

Fig. 3. Association between the acute effect of Δ9 THC on myo-inositol measures in the left Anterior Cingulate Cortex and the changes in cortisol levels post-drug injection. Change in cortisol levels post-drug injection was obtained subtracting cortisol levels 2.5 h post-drug injection from cortisol levels 20 min post-drug injection (20 min post-drug injection minus 2.5 h post-drug injection); the effect of drug administration on change in cortisol levels post-drug injection was obtained subtracting the change in cortisol levels post-drug injection in the placebo condition from the change in cortisol levels post-drug injection in the Δ9-THC condition (Δ9-THC change minus placebo change); the effect of the drug was then correlated with myo-inositol values under Δ9-THC in the whole group by Spearman's correlation; *p* 1-tailed; PLB, placebo Δ9-THC, delta-9-tetrahydrocannabinol.

that loss of glial function may underlie sensitivity to the acute psychotomimetic effects of Δ9-THC, whereas such Δ9-THC-mediated disruption of glial function is absent in subjects not developing psychosis-like symptoms under its influence.

The normal diurnal rhythm of cortisol involves a peak in the levels of the glucocorticoid observed after awakening which then declines progressively (Fries, Dettenborn, & Kirschbaum, 2009). Evidence also suggests that acute Δ-9-THC administration may reduce the normal diurnal decline, resulting in cortisol levels that are either the same as or higher than the baseline (Ranganathan et al., 2009). Results from this study extend such findings, indicating that Δ-9-THC administration may interfere with the normal diurnal decline of cortisol only in those subjects experiencing transient psychotomimetic effects under its influence. In fact, while subjects who did not develop Δ-9-THC-induced psychosis-like symptoms showed a pattern of cortisol decline that was indistinguishable between the Δ-9-THC and placebo conditions, subjects experiencing transient psychotomimetic effects under the influence of Δ-9-THC presented with cortisol levels that were higher than baseline up to 2.5 h following Δ-9-THC exposure, while the same individuals displayed the usual declining pattern under placebo condition. Administration of Δ-9-THC may affect HPA-axis activation by multiple mechanisms. One potential explanation is through activation of presynaptic CB1 receptors on glutamatergic inputs onto corticotrophin-releasing hormone (CRH)-expressing neurons in the paraventricular nucleus of the hypothalamus, in turn affecting glutamate release (Di, Malcher-Lopes, Halmos, & Tasker, 2003; Di, Malcher-Lopes, Marcheselli, Bazan, & Tasker, 2005). Consistent with this, preclinical evidence indicates that high-affinity synthetic CB1 agonists mimic and occlude the inhibitory effect of glucocorticoids on glutamate release, suggesting a modulatory cross-talk between glucocorticoids and endocannabinoids that leads to reduced excitation of the paraventricular nucleus neurons, with relevance to the central control of stress response (Di et al., 2003).

A large body of evidence suggests that glial cells and stress hormones exert synergistic effects and their joint disruption is relevant to several pathological conditions of the central nervous system (Murphy-Royal, Gordon, & Bains, 2019). On one hand, converging evidence from animal and human postmortem studies strongly supports a role of deficits in astrocyte density and function in the limbic regions of the brain in the pathology of stress and glucocorticoid overproduction (Fuchs & Flügge, 2003). On the other, independent preclinical evidence indicates that sustained exposure to stress results in the depletion of gliogenesis in limbic regions including the hippocampus and prefrontal cortex (Czéh et al., 2007). Further, evidence indicates that excessive glutamatergic activity may be deleterious for neurons and contribute to the atrophy of apical dendrites seen under stress conditions in animal models (Conrad, 2006). Astrocytes modulate glutamate availability through the activity of the glial glutamate transporter (GLT-1) and enzyme glutamine synthetase (GS) (Vardimon, Ben-Dror, Avisar, Oren, & Shiftan, 1999). Complementing such evidence, additional studies indicate that glial GLT-1 (Zschocke et al., 2005) and GS (Vardimon et al., 1999) are highly regulated by glucocorticoids and affected by chronic stress (Autry et al., 2006; Reagan et al., 2004). Glucocorticoids also inhibit glucose uptake in the brain, whose energy is essential to the costly task of high-affinity glutamate reuptake (Ritchie, De Butte, & Pappas, 2004), potentially increasing the vulnerability to glutamatergic dysfunction (Li, Yang, & Lin, 2018). Altogether, evidence suggests a complex relationship between glucocorticoids and glia, with implications for the glutamate signaling (Jauregui-Huerta et al., 2010) and the bidirectional communications between neurons and glia (Hinwood, Morandini, Day, & Walker, 2012). Neuron–glia interactions are critical for preserving the homeostatic environment in the central nervous system (Szepesi, Manouchehrian, Bachiller, & Deierborg, 2018) and evidence suggests that they are disrupted in psychiatric disorders such as schizophrenia (Laskaris et al., 2016). Our results extend such evidence, indicating a relationship between the severity of the disrupting effects of Δ9-THC on glial function and cortisol diurnal variations underlying sensitivity to the psychotomimetic effects of the drug.

We employed a study design that allowed us to avoid the confounding effect of cannabis withdrawal, dependence, or intoxication, as we only recruited participants with a lifetime history of minimal cannabis use, abstinent from cannabis for a minimum

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period of 6 months prior to visit, and with a negative urine drug screen for the presence of Δ9-THC at the time of the visit. As all participants had also a lifetime history of negligible use of other common substances of abuse including alcohol, tobacco, and stimulants, it is unlikely that the observed effects on glia and cortisol are attributable to them. Moreover, a minimum interval of 14 days between the two study visits enabled us to avoid any potential carryover effects of Δ9-THC, whose elimination half-life ranges between 18 h and 4.3 days (Kelly & Jones, 1992). However, a number of limitations also needs to be considered. Foremost, caution is warranted in light of the modest sample size studied here. Further, out of a total of 16 participants, only five were deemed as not sensitive to the acute psychotomimetic effects of Δ9-THC. However, we employed a priori previously reported criteria (Bhattacharyya et al., 2018) to determine sensitivity to the psychotomimetic effects of Δ9-THC. Nevertheless, we were able to identify significant differences in Δ9-THC-induced changes in myo-inositol and cortisol levels in minimal cannabis users sensitive to the acute and transient psychotomimetic effects of Δ9-THC compared to those not sensitive, using a within-subject repeated measures experimental design. The strict inclusion criteria, while being advantageous in terms of a controlled sample, also warrant caution in terms of generalizability of the results of the study to the wider context of recreational cannabis use. Similarly, administering Δ9-THC intravenously to study participants allowed much more consistent inter-individual Δ9-THC blood levels compared to other routes of administration (D'Souza et al., 2004), but may affect the generalizability of these results to the effects of recreational cannabis use whose main routes of administration are smoking, inhaling, and swallowing (Baggio et al., 2014). Further, even though evidence supports relatively high reproducibility and test-retest reliability of measures of neurochemicals such as glutamate and myo-inositol at 3 Tesla in healthy subjects (Gasparovic et al., 2011), we did not examine test-retest reliability of the MRS measures for the regions investigated within the context of the present study. However, using a within-subject design allowed us to avoid the confounding effect of between-subject differences in neurochemicals as well as cortisol measures.

In conclusion, it is likely that the transient psychosis-like effects of Δ9-THC are the result of its effects not only on glutamate signaling (Colizzi et al., 2019a) but also differential effects linked to sensitivity to acute psychotomimetic effects on other neurobiological systems including glial function and stress response (Colizzi & Bhattacharyya, 2018), in line with the evidence for multiple neurobiological abnormalities accounting for the complex symptom profile of psychosis (Lisman et al., 2008). In this regard, Δ9-THC-induced abnormalities may be interrelated, with the Δ9-THC-induced glial loss, indexed as myo-inositol reduction, correlating with the perturbation of the normal diurnal decline in cortisol levels. Future studies in larger samples are needed to systematically examine such relationships as well as to replicate the present results. Further, whether differential sensitivity to the effects of Δ9-THC or cannabis use on glial function and neuroendocrine markers linked to the stress response also underlie differential sensitivity to the association between psychotic disorder and regular cannabis use remains to be tested. Future multimodal neuroimaging studies, potentially incorporating a more direct measure of glial function as indexed using Positron Emission Tomography imaging, therefore need to integrate longitudinal information to track the trajectory of change in the bidirectional neuron-glia communication as well as other potential biomarkers associated with cannabis use and examine the relationship of such change with adverse mental health outcomes such as psychosis associated with cannabis use. Elucidating the astrocyte–glucocorticoid interaction and its role in the glutamate signaling may be important in comprehending how the defects in the bidirectional neuron-glia communication could contribute to cannabis-associated psychosis as well as neuropsychiatric conditions developed in response to other environmental or genetic insults.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291720003827

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Clinical trial registration. The present study is not a clinical trial of an IMP; it is a non-therapeutic mechanistic study performed among healthy volunteers to better understand human pathophysiology with reference to the effect of cannabis psychoactive ingredient delta-9-tetrahydrocannabinol (Δ9-THC).

References

Appiah-Kusi, E., Leyden, E., Parmar, S., Mondelli, V., McGuire, P., & Bhattacharyya, S. (2016). Abnormalities in neuroendocrine stress response in psychosis: The role of endocannabinoids. *Psychological Medicine*, *46*(1), 27–45. doi: 10.1017/S0033291715001786

Appiah-Kusi, E., Petros, N., Wilson, R., Colizzi, M., Bossong, M. G., Valmaggia, L., … Bhattacharyya, S. (2020). Effects of short-term cannabidiol treatment on response to social stress in subjects at clinical high risk of developing psychosis. *Psychopharmacology* (*Berl*), *237*(4), 1121–1130. doi: 10.1007/s00213-019-05442-6.

Autry, A. E., Grillo, C. A., Piroli, G. G., Rothstein, J. D., McEwen, B. S., & Reagan, L. P. (2006). Glucocorticoid regulation of GLT-1 glutamate transporter isoform expression in the rat hippocampus. *Neuroendocrinology*, *83*(5–6), 371–379. doi: 10.1159/000096092

Baggio, S., Deline, S., Studer, J., Mohler-Kuo, M., Daeppen, J. B., & Gmel, G. (2014). Routes of administration of cannabis used for nonmedical purposes and associations with patterns of drug use. *Journal of Adolescent Health*, *54*(2), 235–240. doi: 10.1016/j.jadohealth.2013.08.013

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J. A., Kambeitz, J., Prata, D., … McGuire, P. K. (2012). Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and

striatal function. *Molecular Psychiatry*, *17*(12), 1152–1155. doi: 10.1038/mp.2011.187

Bhattacharyya, S., Sainsbury, T., Allen, P., Nosarti, C., Atakan, Z., Giampietro, V., … McGuire, P. K. (2018). Increased hippocampal engagement during learning as a marker of sensitivity to psychotomimetic effects of delta-9-THC. *Psychological Medicine*, *48*(16), 2748–2756. doi: 10.1017/S0033291718000387.

Blest-Hopley, G., O'Neill, A., Wilson, R., Giampietro, V., Lythgoe, D., Egerton, A., & Bhattacharyya, S. (2019). Adolescent-onset heavy cannabis use associated with significantly reduced glial but not neuronal markers and glutamate levels in the hippocampus. *Addiction Biology*, e12827. doi: 10.1111/adb.12827

Bonnefil, V., Dietz, K., Amatruda, M., Wentling, M., Aubry, A. V., Dupree, J. L., … Liu, J. (2019). Region-specific myelin differences define behavioral consequences of chronic social defeat stress in mice. *Elife*, *8*, e40855. doi: 10.7554/eLife.40855.

Borges, S., Gayer-Anderson, C., & Mondelli, V. (2013). A systematic review of the activity of the hypothalamic-pituitary-adrenal axis in first episode psychosis. *Psychoneuroendocrinology*, *38*(5), 603–611. doi: 10.1016/j.psyneuen.2012.12.025

Chen, R., Zhang, J., Fan, N., Teng, Z. Q., Wu, Y., Yang, H., … Chen, C. (2013). Delta9-THC-caused synaptic and memory impairments are mediated through COX-2 signaling. *Cell*, *155*(5), 1154–1165. doi: 10.1016/j.cell.2013.10.042

Colizzi, M., & Bhattacharyya, S. (2018). Neurocognitive effects of cannabis: Lessons learned from human experimental studies. *Progress in Brain Research*, *242*, 179–216. doi: 10.1016/bs.pbr.2018.08.010

Colizzi, M., & Bhattacharyya, S. (2020). Is there sufficient evidence that cannabis use is a risk factor for psychosis? In A. D. Thompson, & M. R. Broome (Eds.), *Risk factors for psychosis: Paradigms, mechanisms, and prevention* (pp. 305–331). Cambridge, Massachusetts: Academic Press.

Colizzi, M., McGuire, P., Pertwee, R. G., & Bhattacharyya, S. (2016). Effect of cannabis on glutamate signalling in the brain: A systematic review of human and animal evidence. *Neuroscience and Biobehavioral Reviews*, *64*, 359–381. doi: 10.1016/j.neubiorev.2016.03.010

Colizzi, M., Weltens, N., McGuire, P., Lythgoe, D., Williams, S., Van Oudenhove, L., & Bhattacharyya, S. (2019a). Delta-9-tetrahydrocannabinol increases striatal glutamate levels in healthy individuals: Implications for psychosis. *Molecular Psychiatry*. doi: 10.1038/s41380-019-0374-8

Colizzi, M., Weltens, N., McGuire, P., Van Oudenhove, L., & Bhattacharyya, S. (2019b). Descriptive psychopathology of the acute effects of intravenous Delta-9-tetrahydrocannabinol administration in humans. *Brain Sciences*, *9*(4). doi: 10.3390/brainsci9040093

Conrad, C. D. (2006). What is the functional significance of chronic stress-induced CA3 dendritic retraction within the hippocampus? *Behavioral and Cognitive Neuroscience Reviews*, *5*(1), 41–60. doi: 10.1177/1534582306289043

Cservenka, A., Lahanas, S., & Dotson-Bossert, J. (2018). Marijuana Use and hypothalamic-pituitary-adrenal axis functioning in humans. *Frontiers in Psychiatry*, *9*, 472. doi: 10.3389/fpsyt.2018.00472

Czéh, B., Müller-Keuker, J. I., Rygula, R., Abumaria, N., Hiemke, C., Domenici, E., & Fuchs, E. (2007). Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: Hemispheric asymmetry and reversal by fluoxetine treatment. *Neuropsychopharmacology*, *32*(7), 1490–1503. doi: 10.1038/sj.npp.1301275

Das, T. K., Dey, A., Sabesan, P., Javadzadeh, A., Théberge, J., Radua, J., & Palaniyappan, L. (2018). Putative astroglial dysfunction in schizophrenia: A meta-analysis of. *Frontiers in Psychiatry*, *9*, 438. doi: 10.3389/fpsyt.2018.00438.

Di, S., Malcher-Lopes, R., Halmos, K. C., & Tasker, J. G. (2003). Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: A fast feedback mechanism. *Journal of Neuroscience*, *23*(12), 4850–4857.

Di, S., Malcher-Lopes, R., Marcheselli, V. L., Bazan, N. G., & Tasker, J. G. (2005). Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and gamma-aminobutyric acid inputs to hypothalamic magnocellular neurons. *Endocrinology*, *146*(10), 4292–4301. doi: 10.1210/en.2005-0610

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., … Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: Implications for psychosis. *Neuropsychopharmacology*, *29*(8), 1558–1572. doi: 10.1038/sj.npp.1300496

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): Facts and future directions. *International Journal of Psychophysiology*, *72*(1), 67–73. doi: 10.1016/j.ijpsycho.2008.03.014

Fuchs, E., & Flügge, G. (2003). Chronic social stress: Effects on limbic brain structures. *Physiology & Behavior*, *79*(3), 417–427. doi: 10.1016/s0031-9384(03)00161-6

Fulford, D., Pearson, R., Stuart, B. K., Fisher, M., Mathalon, D. H., Vinogradov, S., & Loewy, R. L. (2014). Symptom assessment in early psychosis: The use of well-established rating scales in clinical high-risk and recent-onset populations. *Psychiatry Research*, *220*(3), 1077–1083. doi: 10.1016/j.psychres.2014.07.047

Garcia-Segura, L. M., & McCarthy, M. M. (2004). Minireview: Role of glia in neuroendocrine function. *Endocrinology*, *145*(3), 1082–1086. doi: 10.1210/en.2003-1383

Gasparovic, C., Bedrick, E. J., Mayer, A. R., Yeo, R. A., Chen, H., Damaraju, E., … Jung, R. E. (2011). Test-retest reliability and reproducibility of short-echo-time spectroscopic imaging of human brain at 3 T. *Magnetic Resonance in Medicine*, *66*(2), 324–332. doi: 10.1002/mrm.22858

Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., … Zhang, X. (2012). Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell*, *148*(5), 1039–1050. doi: 10.1016/j.cell.2012.01.037

Hillard, C. J., Beatka, M., & Sarvaideo, J. (2016). Endocannabinoid signaling and the hypothalamic-pituitary-adrenal axis. *Comprehensive Physiology*, *7*(1), 1–15. doi: 10.1002/cphy.c160005

Hinwood, M., Morandini, J., Day, T. A., & Walker, F. R. (2012). Evidence that microglia mediate the neurobiological effects of chronic psychological stress on the medial prefrontal cortex. *Cerebral Cortex*, *22*(6), 1442–1454. doi: 10.1093/cercor/bhr229

Jauregui-Huerta, F., Ruvalcaba-Delgadillo, Y., Gonzalez-Castañeda, R., Garcia-Estrada, J., Gonzalez-Perez, O., & Luquin, S. (2010). Responses of glial cells to stress and glucocorticoids. *Current Immunology Reviews*, *6*(3), 195–204. doi: 10.2174/157339510791823790

Kay, S. R., Fiszbein, A., & Opler, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin*, *13*(2), 261–276.

Kelly, P., & Jones, R. T. (1992). Metabolism of tetrahydrocannabinol in frequent and infrequent marijuana users. *Journal of Analytical Toxicology*, *16*(4), 228–235.

Kurosinski, P., & Götz, J. (2002). Glial cells under physiologic and pathologic conditions. *Archives of Neurology*, *59*(10), 1524–1528. doi: 10.1001/archneur.59.10.1524

Laskaris, L. E., Di Biase, M. A., Everall, I., Chana, G., Christopoulos, A., Skafidas, E., … Pantelis, C. (2016). Microglial activation and progressive brain changes in schizophrenia. *British Journal of Pharmacology*, *173*(4), 666–680. doi: 10.1111/bph.13364

Li, C. T., Yang, K. C., & Lin, W. C. (2018). Glutamatergic dysfunction and glutamatergic compounds for Major psychiatric disorders: Evidence from clinical neuroimaging studies. *Frontiers in Psychiatry*, *9*, 767. doi: 10.3389/fpsyt.2018.00767

Lisman, J. E., Coyle, J. T., Green, R. W., Javitt, D. C., Benes, F. M., Heckers, S., & Grace, A. A. (2008). Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends in Neurosciences*, *31*(5), 234–242. doi: 10.1016/j.tins.2008.02.005

Mason, N., Theunissen, E., Hutten, N., Tse, D., Toennes, S., Stiers, P., & Ramaekers, J. (2019). Cannabis induced increase in striatal glutamate associated with loss of functional corticostriatal connectivity. *European Neuropsychopharmacology*, *29*(2), 247–256. doi: 10.1016/j.euroneuro.2018.12.003

Murphy-Royal, C., Gordon, G. R., & Bains, J. S. (2019). Stress-induced structural and functional modifications of astrocytes-further implicating glia in the central response to stress. *Glia*, *67*(10), 1806–1820. doi: 10.1002/glia.23610

Pagotto, U., Marsicano, G., Cota, D., Lutz, B., & Pasquali, R. (2006). The emerging role of the endocannabinoid system in endocrine regulation

https://doi.org/10.1017/S0033291720003827 Published online by Cambridge University Press

and energy balance. *Endocrine Reviews*, *27*(1), 73–100. doi: 10.1210/er.2005-0009

Pearson-Leary, J., Osborne, D. M., & McNay, E. C. (2015). Role of Glia in stress-induced enhancement and impairment of memory. *Frontiers in Integrative Neuroscience*, *9*, 63. doi: 10.3389/fnint.2015.00063

Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *British Journal of Pharmacology*, *153*(2), 199–215. doi: 10.1038/sj.bjp.0707442

Prescot, A. P., Locatelli, A. E., Renshaw, P. F., & Yurgelun-Todd, D. A. (2011). Neurochemical alterations in adolescent chronic marijuana smokers: A proton MRS study. *Neuroimage*, *57*(1), 69–75. doi: 10.1016/j.neuroimage.2011.02.044

Radhakrishnan, R., Wilkinson, S. T., & D'Souza, D. C. (2014). Gone to pot - a review of the association between cannabis and psychosis. *Frontiers in Psychiatry*, *5*, 54. doi: 10.3389/fpsyt.2014.00054

Ranganathan, M., Braley, G., Pittman, B., Cooper, T., Perry, E., Krystal, J., & D'Souza, D. C. (2009). The effects of cannabinoids on serum cortisol and prolactin in humans. *Psychopharmacology* (*Berl*), *203*(4), 737–744. doi: 10.1007/s00213-008-1422-2

Reagan, L. P., Rosell, D. R., Wood, G. E., Spedding, M., Muñoz, C., Rothstein, J., & McEwen, B. S. (2004). Chronic restraint stress up-regulates GLT-1 mRNA and protein expression in the rat hippocampus: Reversal by tianeptine. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(7), 2179–2184. doi: 10.1073/pnas.0307294101

Ritchie, L. J., De Butte, M., & Pappas, B. A. (2004). Chronic mild stress exacerbates the effects of permanent bilateral common carotid artery occlusion on CA1 neurons. *Brain Research*, *1014*(1–2), 228–235. doi: 10.1016/j.brainres.2004.04.036

Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Viganò, D., … Parolaro, D. (2009). Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus*, *19*(8), 763–772. doi: 10.1002/hipo.20554

Sami, M. B., Rabiner, E. A., & Bhattacharyya, S. (2015). Does cannabis affect dopaminergic signaling in the human brain? A systematic review of evidence to date. *European Neuropsychopharmacology*, *25*(8), 1201–1224. doi: 10.1016/j.euroneuro.2015.03.011

Schoeler, T., Monk, A., Sami, M. B., Klamerus, E., Foglia, E., Brown, R., … Bhattacharyya, S. (2016a). Continued versus discontinued cannabis use in patients with psychosis: A systematic review and meta-analysis. *The Lancet. Psychiatry*, *3*(3), 215–225. doi: 10.1016/S2215-0366(15)00363-6

Schoeler, T., Petros, N., Di Forti, M., Pingault, J. B., Klamerus, E., Foglia, E., … Bhattacharyya, S. (2016b). Association between continued Cannabis use and risk of relapse in first-episode psychosis: A quasi-experimental investigation within an observational study. *JAMA Psychiatry*, *73*(11), 1173–1179. doi: 10.1001/jamapsychiatry.2016.2427

Stella, N. (2010). Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia*, *58*(9), 1017–1030. doi: 10.1002/glia.20983

Szepesi, Z., Manouchehrian, O., Bachiller, S., & Deierborg, T. (2018). Bidirectional microglia-neuron communication in health and disease. *Frontiers in Cellular Neuroscience*, *12*, 323. doi: 10.3389/fncel.2018.00323

Vardimon, L., Ben-Dror, I., Avisar, N., Oren, A., & Shiftan, L. (1999). Glucocorticoid control of glial gene expression. *Journal of Neurobiology*, *40*(4), 513–527. doi: 10.1002/(sici)1097-4695(19990915)40:4<513::aid-neu8>3.0.co;2-d

Verkhratsky, A., Steardo, L., Parpura, V., & Montana, V. (2016). Translational potential of astrocytes in brain disorders. *Progress in Neurobiology*, *144*, 188–205. doi: 10.1016/j.pneurobio.2015.09.003

Zschocke, J., Bayatti, N., Clement, A. M., Witan, H., Figiel, M., Engele, J., & Behl, C. (2005). Differential promotion of glutamate transporter expression and function by glucocorticoids in astrocytes from various brain regions. *Journal of Biological Chemistry*, *280*(41), 34924–34932. doi: 10.1074/jbc.M502581200

https://doi.org/10.1017/S0033291720003827 Published online by Cambridge University Press